REVISITING STREAMSIDE TREE WATER USE IN THE CONTEXT

OF THE TWO WATER WORLDS HYPOTHESIS

by

Emily Suzanne Schulze

A thesis submitted to the faculty of
The University of Utah
in partial fulfillment of the requirements for the degree of

Master of Science

Department of Biology

The University of Utah

August 2015

The University of Utah Graduate School

STATEMENT OF THESIS APPROVAL

The thesis of **Emily Suzanne Schulze**

has been approved by the following supervisory committee members:

David R. Bowling	, Chair	**June 10, 2015** Date Approved
John Sperry	, Member	**June 10, 2015** Date Approved
Douglas Kip Solomon	, Member	**June 10, 2015** Date Approved

and by **Denise Dearing** , Chair/Dean of

the Department/College/School of **Biology**

and by David B. Kieda, Dean of The Graduate School.

ABSTRACT

Previous stable isotope research identified a riparian meadow in the Rocky Mountains where streamside box elder (*Acer negundo)* trees did not use stream water, the most reliable and readily available source. Further dual isotope analysis showed that the water used by trees appeared to be more evaporatively enriched than all available measured sources, including stream water, precipitation-derived soil water, and groundwater. While it is unlikely that there is a missing pool of water accessed by the trees, they may be tapping into a distinct subset of the bulk soil water available, possibly derived from snowmelt from the preceding winter. In this study, we investigated whether snowpack sublimation and melt may impart an enriched isotopic signature that persists throughout the following growing season in less-mobile soil water pools. Depth profiles of snow, bulk melt water, and early season soil lysimeter water were collected throughout the winter and analyzed for hydrogen and oxygen stable isotopes. As snow began to melt in the spring, water samples for isotope analysis were taken from soil profiles, stream water, groundwater, and stems. Although sublimation may have occurred at the site, neither sublimation nor exchange between melt water and snow imparted an evaporative isotope enrichment signal on the snowpack. However, tree xylem water remained isotopically enriched relative to all sources during the following growing season. These findings suggest that snowpack sublimation and liquid-ice isotopic exchange are not the source of evaporative enrichment in tree xylem water.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to my thesis committee, Dr. Dave Bowling (chair), Dr. John Sperry, and Dr. Kip Solomon, for their guidance, support, and willingness to listen to my crazy brainstorming. Thank you to the Bowling and Pataki labs for all of their feedback through the years. I am grateful to Suvankar Chakraborty, Lori Long, and the SIRFER lab for all of their assistance in water extractions and analysis. I would also like to acknowledge the Global Change and Sustainability Center and iUTAH for their financial support and collaborative opportunities. I wish to express my sincere thanks to my countless field partners: Allison Chan, Dave Eiriksson, La' Shaye Ervin, Steven Hall, and Simone Jackson - I probably would have been eaten by a bear if it were not for your company. Thank you also to Christy Mancuso, La' Shaye Ervin, Allison Chan, and Bob Cieri for editing a mess of first drafts – I'm sure it was exhausting work.

Thank you to my parents, John and Carla Breiner, for nudging me towards a career in science years ago, and to my sisters, Anna Clements and Megan Breiner; scientists who continue to inspire me and make me work harder. Finally, I would like to offer my deepest gratitude to my husband Peter: field partner, life partner, and supporter of my dreams. Your encouragement kept me motivated and your patience kept me sane – I could not have done this without you.

INTRODUCTION

Naturally abundant water stable isotopes act as natural tracers that are both identifiable and quantifiable as they move from source to sink. Importantly, changes in the isotopic ratios of hydrogen and oxygen can indicate physical processes like precipitation, evaporation, and sublimation. These intrinsic traits of stable isotopes have led to their common usage in the field of ecohydrology. In recent decades, stable isotope approaches have been used to address questions of water source partitioning [Weltzin and McPherson 1997, Jackson et al. 1999, Meinzer et al. 1999], seasonal patterns of water use [Dawson and Pate 1996, Zencich et al. 2002], and landscape level water budgets [Ehleringer et al. 2000, Dawson et al. 2002].

The examination of rooting depth and plant water uptake is a common application of water stable isotopes because it is useful for determining ecosystem response to water availability [Bertrand et al. 2014]. Several studies from the late 20th century relied on either the ratio of hydrogen (δ^2H) or oxygen ($\delta^{18}O$) isotopes to indicate active rooting depths for plants. Stable isotope measurements are relatively noninvasive in that they do not require root excavation and destruction, and they extend measurements beyond one snapshot in time [Ehleringer and Dawson 1992]. It has been shown that plants do not fractionate water isotopes during uptake from the roots [White et al. 1985, Dawson and Ehleringer 1991] with the exceptions of halophytes and some woody xeriphytes [Dawson and Ehleringer 1991, Ellsworth and Williams 2011]. Thus the simple comparison of stem xylem water to vertical profiles of soil water, to streams, and to shallow groundwater reveal plant water use patterns [Allison et al. 1984].

In a landmark study, Dawson and Ehleringer [1991] used hydrogen isotopes to show that streamside trees were not using stream water, the most readily available source. Although intuition suggests trees with roots growing directly into a small mountain stream would be accessing stream water, the hydrogen isotopes did not match. The authors suggested the trees were instead using some deeper groundwater source. In this study, we revisit the study location of Dawson and Ehleringer [1991], now analyzing water from many of their study trees for dual-isotopes (δ^2H and $\delta^{18}O$) and incorporating samples of groundwater from new wells. Results from a sampling campaign in 2004 confirm Dawson and Ehleringer's conclusion: the trees are not relying on stream water. However, extracted water from varying depths of soils and groundwater did not explain the persistent isotopic enrichment of trees either (Figure 1). Note that a line through the water from the stems had a shallower slope than that of soil water, stream water, and groundwater, suggesting that the trees were obtaining water from an unidentified pool or combination of pools.

While it is unlikely that there is some "missing" water resource available to trees, it is possible that smaller, distinct pools exist within the soil. Recently, scientists have suggested a "Two Water Worlds Hypothesis" in which at least two separate water pools exist in soils: a mobile pool and a second tightly-sorbed, less-mobile pool [Brooks 2010, Bertrand 2014, McDonnell 2014]. Showcased in Brooks et al. [2010], the first water to enter a dry soil will tightly sorb to soil particles. At Brooks' site in Oregon, this wetting event was the first autumn rain after a long, dry summer. The rain from this storm created a Rayleigh distillation outline through

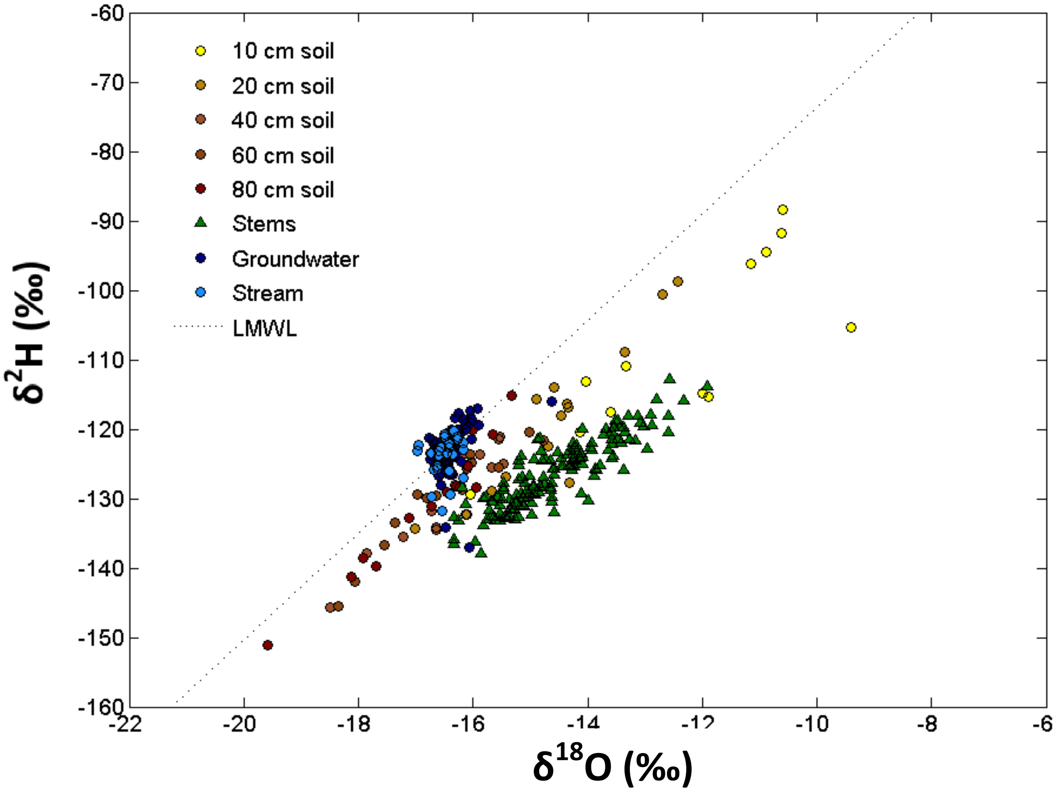

Figure 1: Stable isotope composition of water collected/extracted from soil cores at depths from 10-80 cm, stems of trees, adjacent groundwater wells, and a nearby stream from June - September 2004, relative to the local meteoric water line (LMWL).

the soil profile, with the most enriched (heaviest) water precipitating from the atmosphere first and thus adhering to the topmost soil particles. Tightly-sorbed water deeper in the soil became more and more depleted (lighter) as the storm moved over the land surface. Subsequent rain events continued to move via typical translatory flow, pushing water through the profile and eventually into groundwaters and streams. However, the tightly-sorbed soil water from the first rain event did not mix with subsequent water, and persisted throughout the warm season to later be used by trees when mobile water was absent.

At our study site just outside of Salt Lake City, UT, snowmelt is the dominant water input after a dry and cold winter season, rather than an autumn storm [Maurer and Bowling, 2014]. Therefore, the tightly-sorbed, immobile pool of water should isotopically reflect the first snow melt event. If this tightly-sorbed soil water is indeed the source of water for trees, wintertime snowpack metamorphism from water vapor diffusion, sublimation, or liquid-ice exchange may change the isotopic signature of the snow, shifting it below the local meteoric water line (LMWL).

Snow retains a depleted isotopic signature that is consistent with the atmosphere in which it was formed in the clouds, unlike rain which exchanges with humidity near the land surface as it falls [Gat 1996]. When plotted in dual-isotope space, snow precipitation is therefore isotopically depleted in both $\delta^{2}H$ and $\delta^{18}O$ relative to rain but still remains along the meteoric water line (Figure 2a). One might expect that sublimation would not have any overall isotopic fractionating effect if entire snow surface layers are removed without mass dependent discrimination [Friedman et al. 1991]. However, through the winter the accumulated snowpack can be altered by a number of processes, namely water

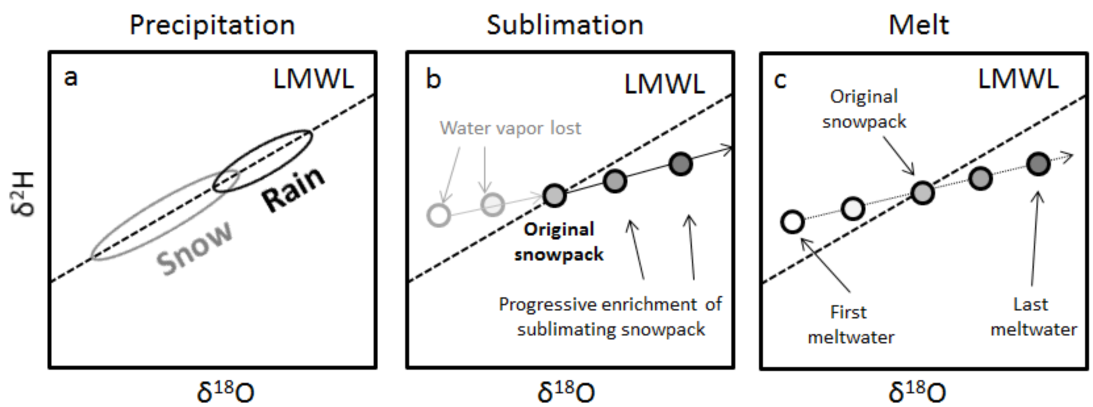

Figure 2: Conceptual diagrams of snowpack isotopic evolution. (a) Precipitation falling as snow is more depleted than rain and falls along the local meteoric water line (LMWL). (b) A snowpack undergoing sublimation vapor loss becomes progressively enriched as the fraction of ice remaining decreases (direction of arrow). (c) The first meltwater to leave a snowpack is isotopically depleted, while the snowpack becomes progressively enriched below the LMWL as fraction of ice remaining decreases (direction of arrow). Figure adapted from Clark and Fritz [1997].

vapor diffusion and melting with subsequent interaction with ice [Moser and Stichler 1974, Taylor et al. 2001, Earman et al. 2006, Zhou et al. 2008].

Water vapor in snowpack pores is isotopically depleted relative to the snow crystals at equilibrium because there is a difference in vapor pressures of $H_2{}^{18}O$ and ^2HHO that imparts a disproportional enrichment of heavy isotopologues in the solid phase during sublimation. This depleted water vapor can then diffuse along temperature-induced vapor pressure gradients throughout the snowpack, mixing with isotopically distinct layers and recondensing in new layers to ultimately create a heterogeneous isotope profile [Colbeck 1991, Taylor et al. 2001]. Additionally, liquid water from melting snow at the pack surface will exchange with water vapor within snowpack pores as it percolates throughout the pack, again altering isotopic profiles [Taylor et al. 2001]. When the conditions for sublimation occur (high solar radiative input, high winds), and snow is removed from at and near the snow surface, the remaining pack is not necessarily representative of freshly fallen snow [Sokratov and Golubev 2009]. Several laboratory and field studies have shown a progressive isotopic enrichment as snowpacks sublimate and lose mass [Moser and Stichler 1974, Kendall and McDonnell 1996, Sokratov and Golubev 2009, Gustafson et al. 2009, Biederman et al. 2012], resulting in a snowpack that falls below the LWML (Figure 2b).

In addition to wintertime snowpack metamorphism and sublimation, the gradual springtime snow melting process can also impart isotopic variations over time. Heavier isotopologues of water (^2HHO or $H_2{}^{18}O$) have slightly shorter bonds with stronger bond energies than $H_2{}^{16}O$ [Gat 1981]. As the snow pack begins to melt, the first melt water is relatively depleted in heavier isotopes compared to the remaining pack due to slight differences in bond energies between heavier and lighter water. Several studies have shown that throughout the progression of melt, melt water shifts from being isotopically depleted to isotopically enriched relative to the original snowpack [Taylor et al. 2001, Feng et al. 2002, Gurney and Lawrence 2004]. Importantly, the slope along which this melt process occurs is almost always shallower than the LMWL (Figure 2c) [Zhou et al. 2008, Lee et al. 2010].

In this study, we investigated winter and spring isotopic effects of snowpack sublimation and melt, and whether these isotopic changes in snowpack can explain the apparent enrichment of tree xylem water in the subsequent growing season shown in Figure 1. We hypothesized that the isotopic enrichment signal imparted by snowpack sublimation and melt persists in the first water to infiltrate dry soils at our site, which effectively becomes the less mobile soil water pool as demonstrated by Brooks et al. [2010]. According to the "Two Water Worlds Hypothesis" trees may preferentially extract water from this pool. If so, these wintertime snow processes may explain the apparent enrichment of xylem water relative to bulk soil water at our site in previous years.

METHODS

Site description

Samples were collected from a small mountain meadow (studied by Dawson and Ehleringer [1991]) in Red Butte Canyon, a protected Research Natural Area located in the Wasatch Mountains just east of Salt Lake City, Utah. Local climate is characterized by hot, dry summers and long, cold winters. Mean annual precipitation is 700 mm, falling largely as snow in winter, with sporadic summer rain [Hely et al. 1971, Bond et al. 1977]. The meadow is at ~1800 m elevation and is approximately 1045 m^2, bordered on its western edge by a first-order stream. Stream flow peaks during snowmelt in May where it is often an order of magnitude greater than in September at minimum flow. Although nearly a quarter of the annual precipitation falls as rain in the summer, mean stream flow does not increase during this period [Ehleringer et al. 1992]. Soils in Red Butte Canyon range from sandy to loamy clays, and are neutral to slightly basic with an average depth around 1m, especially along riparian reaches. The dominant woody flora include boxelder (*Acer negundo)*, and bigtooth maple (*Acer gradidentatum)* which grow along the stream and along a hillslope on the eastern boarder of the meadow. The meadow area is dominated by species of *Solidago* and *Poa* grasses.

Snow and snowmelt sampling

The snowpack was measured and sampled monthly from January 2014 until full snow melt in late March 2014 along three ~20 m transects representative of microclimate variability under the tree canopy near the stream, in the open meadow, and under a tree canopy in the meadow. Snow pack cores were sampled monthly in roughly 10 cm depth increments from snow surface to soil surface and transferred to 0.5 L plastic Nalgene bottles. Bottles were sealed with wax film and allowed to melt before subsampling into 20 mL glass vials. Vials were sealed with wax film and stored in a freezer until further analysis. Snow melt was collected in three 5-gallon buckets, positioned in soil pits with openings level with soil surface. A mesh screen covered the opening to prevent bulk snow or organic litter accumulation throughout the winter. A small PVC pipe extended from the bottom of the bucket to breast height for easy access sampling. As snow began to melt, melted water collected in buckets was pumped through the PVC pipe and collected in 100 mL Nalgene plastic bottles. Subsamples were transferred to 20 mL glass vials, sealed with parafilm, and frozen until analysis. Twelve lysimeter bottles were installed at approximately 20 cm depth into meadow soil prior to snow accumulation in a 3x4 grid. Each lysimeter was pumped to -200 kPa in order to access mobile soil water. Accumulated water was subsampled from buried jars into 20 mL vials, sealed with parafilm, and frozen until analysis.

Xylem water sampling

Trees growing along the eastern bank of the stream were sampled monthly from May until September in 2014. Monthly sampling dates on 5/3/14, 5/29/14, 7/1/2014, 7/28/2014, and 9/1/2014 are henceforce referred to as May = 5/3/14, June =

5/29/14 , July = 7/1/2014, August = 7/28/2014, September = 9/1/2014 for ease. Healthy, suberized stems from *Acer negundo* and *Acer grandidentatum* were clipped, stripped of periderm and phloem, and placed inside 20 mL glass vials. These vials were wrapped with wax to prevent evaporative water loss, and refrigerated at 4 °C until analysis.

Stream and groundwater sampling

Stream water was sampled midstream, making sure to sample only freely flowing water. Vials were sealed with parafilm to prevent evaporative fractionation and refrigerated until analysis. Groundwater levels were measured using an electronic water level graduated meter before each purge. At least two casing volumes of water were purged prior to sample collection from each piezometer, using an inertial pump (Solinst Model 404). Sample vials were washed with well water three times prior to final collection.

Soil water sampling

Soil cores were taken in 10 – 20 cm increments to a depth of 100 cm from just below the tree canopy and approximately 5 m away from the stream bank using a ratcheted soil bucket auger. Contents were emptied into a mixing vessel and roughly homogenized by shaking before a representative subsample was transferred to vials. Vials were sealed with parafilm and refrigerated until analysis. Soil moisture sensors (Digital TDT Moisture Sensor, Acclima Inc., Meridian, ID) associated with a local climate station measured soil moisture at depths of 5, 10, 20, 50, and 100 cm approximately 10 m from trees of interest. Similar methods were employed for the 2004 sampling period and the 2014 growing sample collections.

Water extractions

Water was extracted from stem and soil samples according to the cryogenic vacuum distillation method at the University of Utah's Stable Isotope Ratio Facility for Environmental Research (SIRFER) isotope facility [Ehleringer et al. 2000]. Samples were run to full completion (~ 90 minutes) to ensure total transfer of water and inhibit any Rayleigh fractionation effects [West et al. 2006, Orlowski et al. 2013]. Extracted waters were stored with activated charcoal for a minimum of 72 hours to minimize organic compound contamination of water samples prior to mass spectrometry analysis. Groundwaters and stream water were filtered using a filter to remove sediment in water samples prior to analysis. Extracted water samples from 2014 were processed using a Picarro L2130-i Analyzer to measure both hydrogen and oxygen isotope ratios. Samples from 2004 were analyzed by isotope-ratio mass spectrometry (IRMS) at the SIRFER facility and for isotope ratios of hydrogen and oxygen. Isotope ratios are expressed relative to Vienna Standard Mean Ocean Water (VSMOW) in ‰ notation:

$$\delta^{2}H \text{ or } \delta^{18}O = \left(\frac{R_{sample}}{R_{standard}} - 1\right)*1000 \quad [1]$$

where R is the ratio of deuterium (^{2}H) to hydrogen or ^{18}O to ^{16}O atoms of the sample and VSMOW. Instrument precision was 1.1 ‰ and 0.2 ‰ for $\delta^{2}H$ and $\delta^{18}O$, respectively.

Water potential measurements

Tree water potentials were measured at predawn and midday throughout the 2014 growing season using a pressure chamber [Scholander et al. 1965] in order to assess tree water stress.

RESULTS

Snow and snowmelt isotopic evolution

Snow pack accumulated through late March, with a maximum snow depth of 0.57 m on February 2, 2014. All snow water samples (snowpack cores, melt water, and soil lysimeters) fell along the LMWL ($y = 7.67x - 3.1102$) [G Bowen, unpublished results] (Figure 3a). Snowpack and lysimeter samples had slopes that were only slightly shallower than the LMWL (7.4 and 6.9, respectively), while isotopes measurements of snow melt had a substantially shallower slope (4.8) (Table 1). However, snow melt collected on March 15 (latest sampling date) strongly influenced this slope (Figure 3b). Snow waters in dual-isotope space are illustrated in Figure 3a, clearly showing minimal deviation from the LMWL in general.

Although initially depleted, snowmelt progressively enriched over time (Figure 3b). This progressive snow melt enrichment was subsequently manifested in the bulk soil water profile collected following snow melt on March 15 (Figure 3c). The most depleted water was extracted near the soil surface, and the most enriched water was at depth. When plotted in dual-isotope space (δ^2H vs $\delta^{18}O$), this early bulk soil water had a slope of 7.2 (R^2=0.97, n=10), very similar to the LMWL.

Tree and soil water

Similar to the 2004 growing season results, tree extracted water collectively appeared more isotopically enriched (below and right of LMWL) than stream water,

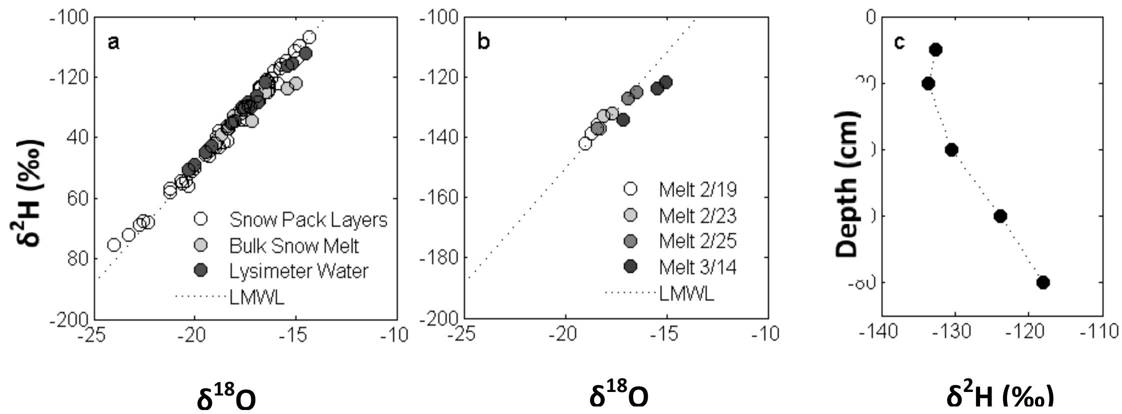

Figure 3: Snowpack and snowmelt isotope data for winter 2014. (a) Isotope ratios of snowpack cores taken in 10 cm increments, bulk snow melt collected in buckets, and snowmelt water collected with tension lysimeters, relative to the LMWL (dashed line) (b) Progression of snowmelt isotope ratios between Feb. 19, 2014 and March 14, 2014 relative to the LMWL (c) Isotopic profile of cryogenically-extracted soil water following the end of the snow melt period on March 15, 2014.

Table 1

Best-fit line slopes of plots of δ^2H vs $\delta^{18}O$ for soil waters, stem waters, and snow waters for 2004 and 2014, with associated R^2 values for sample size (n). LMWL slope [G Bowen, unpublished results] for comparison.

	Slope of best-fit line	δ^2H Offset (‰)	R^2 *(n)*
2004 Soil Water	5.8	-35	0.87 *(76)*
2004 Stem Water	4.7	-59	0.77 *(147)*
2014 Snowpack	7.4	-2	0.99 *(52)*
2014 Snow Melt	4.8	-49	0.90 *(12)*
2014 Lysimeter	6.9	-11	0.99 *(19)*
2014 Soil Water	4.7	-52	0.88 *(81)*
2014 Stem Water	4.1	-71	0.93 *(77)*
LMWL	*7.7*	*-3*	--

groundwater, or soil water throughout the 2014 growing season (Figure 4). Trees began to bud in May, although leaves were not fully expanded until the June sampling date (5/29/14), and senescence occurred in late September. From May to September 2014, tree and soil extracted water fell below the LMWL with slopes of 4.1 (R^2=0.93, n=77) and 4.7 (R^2=0.88, n=81) for trees and soil, respectively. In contrast, all groundwater and stream water cluster together and directly on the LWML throughout the growing season (Figure 4).

A monthly breakdown of trees and soils (Figure 5) revealed certain sampling dates in which trees align with the soil evaporation line (July and Aug) while in other months, the isotopic composition of tree water fell below the soil line at shallower slopes (May, June, Sept) (Table 2). As the trees were just beginning to leaf out during May 2014, the trees appeared the most evaporatively enriched (trees: slope = 3.41, R^2=0.93, n = 10; soils: slope=5.24, R^2=0.95, n =12) and displayed the largest spread of values in dual-isotope space. Once the trees were fully flushed (June 2014), the isotopic composition was more enriched than soil water, although only slightly offset and with a similar slope (trees: slope = 3.72, R^2=0.89, n=10; soil: slope = 5.39, R^2=0.99, n = 12). Conversely, in July and August, extracted tree xylem water fell just along the soil water evaporation/equilibrium line (July trees: slope = 3.00, R^2=0.64, n = 10; soil: slope = 3.36, R^2=0.79, n = 12; August trees: slope = 5.55, R^2=0.85, n = 10; soil: slope = 3.18, R^2=0.95, n = 12). However, September tree water once again fell below soil water at a much shallower slope (trees: slope = 2.90, R^2=0.30, n =10; soil: slope = 5.53, R^2=0.98, n = 12).

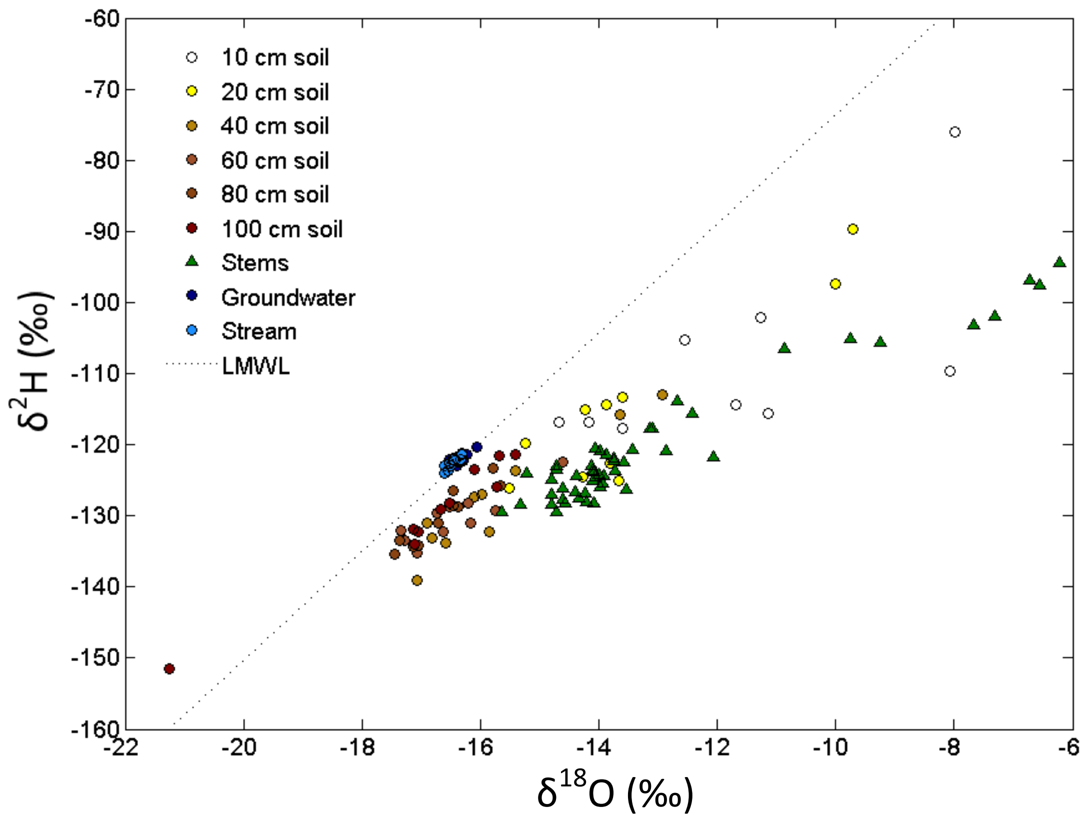

Figure 4: Stable isotope composition of water collected/extracted from soil cores at depths from 10-100 cm, stems of trees, adjacent groundwater wells, and a nearby stream from 2014 growing season, relative to the local meteoric water line [G. Bowen, unpublished data].

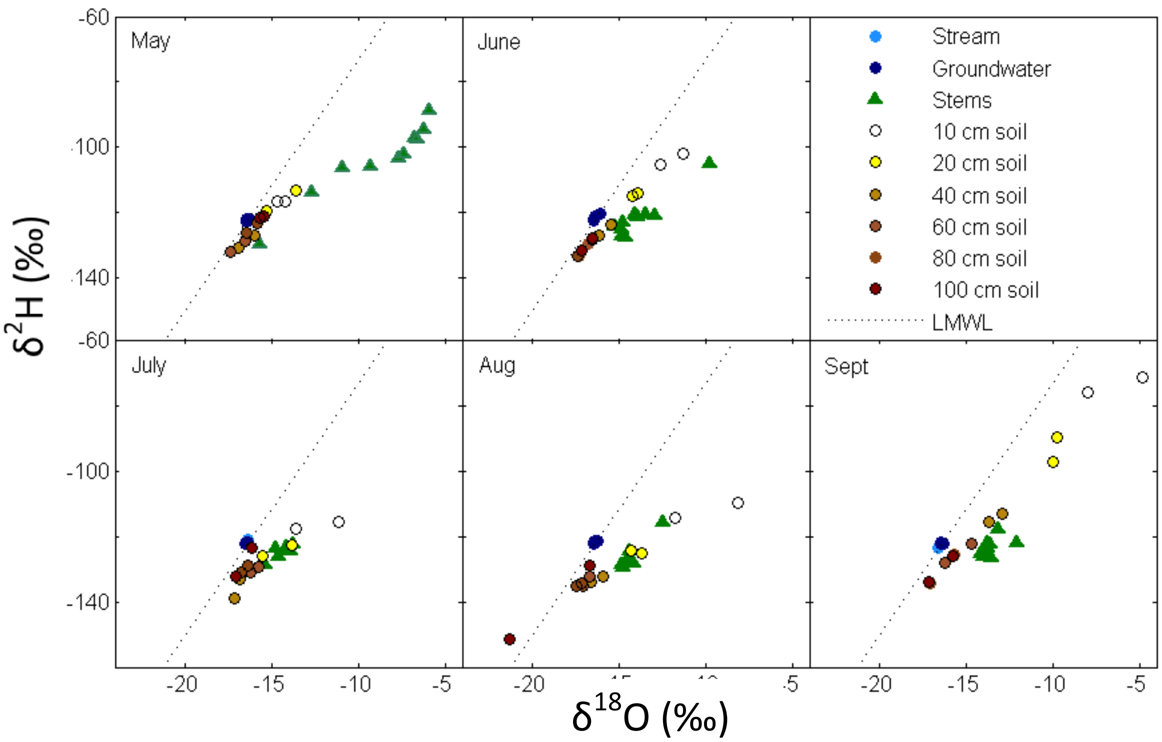

Figure 5: Stable isotope composition of water collected/extracted from soil cores at depths from 10-100 cm, stems of trees, adjacent groundwater wells, and a nearby stream from May - September 2014, relative to the local meteoric water line [Bowen, unpublished data].

Table 2

Monthly best-fit line slopes of plots of δ^2H vs $\delta^{18}O$ for stem waters and soil waters for the growing season 2014, with associated R^2 values for sample size (n).

	Tree Extracted Water			**Soil Extracted Water**		
	Slope of best-fit line	δ^2H Offset (‰)	R^2 *(n)*	Slope of best-fit line	δ^2H Offset (‰)	R^2 *(n)*
May	3.4	-74	0.93 *(10)*	5.2	-41	0.95 *(12)*
June	3.7	-70	0.89 *(10)*	5.4	-39	0.99 *(13)*
July	3.0	-82	0.64 *(10)*	3.4	-75	0.79 *(12)*
Aug	5.6	-47	0.85 *(10)*	3.2	-80	0.95 *(12)*
Sep	2.9	-84	0.30 *(10)*	5.5	-39	0.98 *(12)*

Soil moisture influence

Throughout the growing season, soil volumetric water content (VWC) varied across depth and time, with deep soil moisture below 50 cm gradually declining throughout the season and shallower soil depths responding to summer rains (Figure 6). Throughout this period, tree water potentials were measured and soil water potentials were estimated using midday and predawn water potential measurements (Table 3), with little variability. Additionally, depth to groundwater for each of the 5 wells had little variation throughout the season (Table 4).

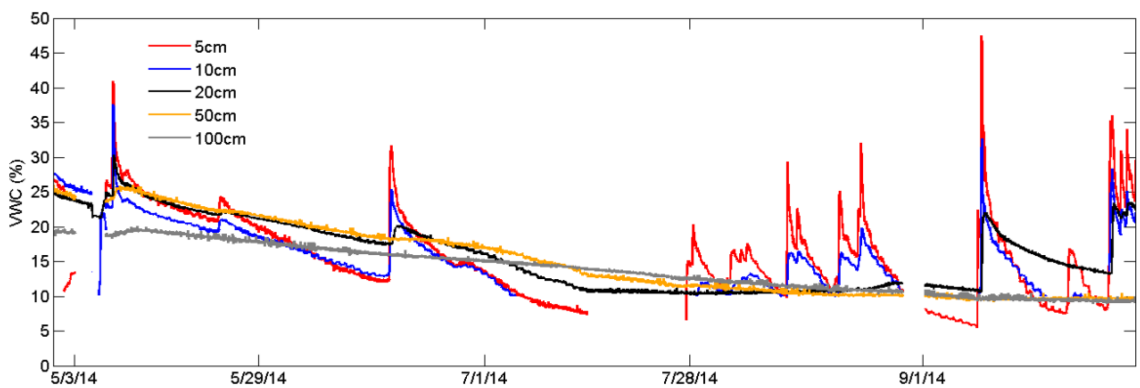

Figure 6: Volumetric water content (VWC) of soils at depths of 5cm, 10cm, 20cm, 50cm, and 100cm during the growing season 2014. Month ticks correspond to the sampling dates for various water isotopes.

Table 3

Average predawn and midday water potentials of trees (n=18) across the 2014 growing season with standard deviations.

	May	June	July	Aug	Sept
Predawn Ψ (MPa)	**-0.78** ± 0.19	**-0.51** ± 0.02	**-0.45** ± 0.04	**-0.38** ± 0.03	**-0.61** ± 0.09
Midday Ψ (MPa)	**-0.82** ± 0.19	**-1.6** ± 0.23	**-1.43** ± 0.32	**-1.74** ± 0.23	**-1.49** ± 0.29

Table 4

Depths of wells installed among trees and along stream with average depth to top of the water column of each well across the 2014 growing season

	Total well depth (m)	Avg. depth to water (m)
Well 1	11.3	7.0 ± 0.4
Well 2	14.9	8.6 ± 0.3
Well 3	9.6	4.5 ± 0.1
Well 4	14.4	7.5 ± 0.3
Well 5	15.2	7.8 ± 0.2

DISCUSSION

Isotopic effect of wintertime snowpack metamorphosis

A number of winter processes can cause fractionation of a snowpack and shift its isotopic composition away from the LMWL, including evaporation/sublimation, melting/refreezing, vapor diffusion, and overall phase changes from ice to liquid water or water vapor. Sublimation and evaporation impart kinetic fractionations that gradually enrich the snowpack, causing a shift to a shallower slope in dual-isotope space [Stichler et al. 2001, Lee et al. 2010]. However, exchange with atmospheric water vapor may cause a shift back towards the LMWL, masking any effect of sublimation [Earman et al. 2006]. The relative importance of these processes is highly variable depending on local climate and microclimate, snowpack structure, humidity, and solar radiation input [Sommerfeld et al. 1987, Earman et al. 2006, Gustafson et al. 2009, Biederman et al. 2012].

In contrast to our expectations, wintertime snow metamorphosis, melt, and sublimation did not appreciably shift the snowpack away from the LMWL at our site. In fact, each snowpack remained clustered around the LMWL throughout the winter. This is likely a result of a shallow snowpack that remains closely coupled to the atmospheric temperature. When snow packs are thick enough to insulate the ground below, the deppest layers of the snowpack are more stable and remain near 0°C compared to those at the snowpack surface that are exposed to fluctuating colder and warmer atmospheric temperatures [Colbeck 1991]. Because the snow pack at our site remained quite shallow (0.57 m at its deepest), water vapor diffusion and mixing through the snowpack may not move primarily upward, as might be expected in deeper snowpacks [Sommerfeld et al. 1987]. Additionally, any snowpack pore water vapor would quickly equilibrate with the surrounding snowpack at the high relative humidity characteristic of snow packs, limiting any mass dependent diffusion gradients [Friedman et al. 1991]. All of this would only be true in the absence of wind, which enhances gas transport out of the snowpack [Bowling and Massman 2011]. Because all layers of the snowpack remained on the LMWL, if sublimation had occurred and removed layers from the surface of the snowpack throughout the winter, this bulk removal would not shift the remaining pack away from the LMWL because this removal is not mass dependent and would not preferentially remove hydrogen over oxygen. This has been found in field studies [Friedman et al. 1991, Dietermann and Weiler 2013], although lab experiments have shown isotopic evolution [Sommerfeld et al. 1987, Stichler et al. 2001].

Although our study did not measure sublimation rate or loss, it is possible sublimation still occurred without affecting the isotopic composition. It is unclear whether sublimation will actually induce kinetic fractionation and impose a shift from the LMWL because it is often considered a minor process in relation to atmospheric water vapor condensation [Friedman et al. 1991, Gustafson 2009]. Nighttime equilibration with atmospheric water vapor has been shown to pull any evaporatively enriched isotope signature back towards the LMWL by morning [Dieterman and Wieler 2013]. Regardless, wintertime processes impacting the snowpack did not shift snowpack isotopes away from the LMWL enough to explain the enrichment of tree water at our site during the growing season.

Isotopic evolution of snow melt

Water collected from snow melt buckets displayed a slight shift away from the LWML as expected with a slope of 4.8 (Table 1, Figure 3a). This shift away is due to the different fractionation factors for solid to liquid transformations compared to the vapor to liquid fractionation factors for precipitation [Lee et al. 2009]. Many have reported much shallower snow melting slopes compared to the global meteoric water line (GMWL) slope of 8 [Taylor et al. 2001, Feng et al. 2002, Gurney and Lawrence 2004, Lee et al. 2010]. However, in our study, the snowpack melted rapidly, which could account for the only slight deviation from the LMWL in dual-isotope space.

While many argue that the aforementioned fractionation factors cause the shallow slope shift of melting snow, Earman et al. [2006] claim greater exposure times are responsible for increased enrichment in later meltwaters, as demonstrated by a time-integrated total snowmelt isotopic signature that was more enriched than original bulk snowpack. If this is indeed the case, the rapid melting of snow would minimize any 'exposure-time' differences between first meltwater and last meltwater. Likely, the gradual enrichment of snow meltwater is firstly directed by the fractionation factors associated with phase change, and can be enhanced by later evaporation/sublimation/vapor exchange from longer exposure times.

Although minor, progressive enrichment of heavy isotopes was detected over time in melt water (Figure 3b). According to our prediction, the first water to melt from the snowpack is depleted relative to the bulk pack (Figure 2c) [Clark and Fritz 1997]. In accordance with the results of Brooks et al. (2010), this first water to enter the winter-dried soil adhered to soil particles and filled the smallest soil pores at the surface. As the snow continued to melt and infiltrating melt water gradually became more enriched, small pores within the soil matrix were filled before allowing subsequent water to pass through. Our soil core water extractions following snow melt in March clearly show this progression, setting up an isotopic profile in the soil in the same manner as Brooks et al. (2010), although in the opposite qualitative pattern compared to their rainfall-based profile (heaviest at depth in our case rather than near the soil surface as in theirs).

It is interesting to note that although total snowmelt deviated from the LMWL, the lysimeter water collected directly after snow melt remained along the LMWL. Because lysimeter water is considered the more "mobile" pool of water, this also supports "Two Water Worlds Hypothesis" in which mobile soil water appears isotopically similar to stream water, while soil matrix bound water and trees fall off of the LMWL [Brooks et al. 2010, McDonnell 2014].

Enrichment of tree xylem water

The isotopic composition of tree xylem water remained evaporatively enriched relative to other water sources throughout the 2014 growing season. Because we did not detect substantial fractionation from snow sublimation or melt, these wintertime processes do not explain the persistent isotopic enrichment of tree water throughout the following growing season. Indeed, results from the 2014 growing season further support the notion that the results from 2004 were not merely anomalies, demonstrating the continued pattern of a shallower δ^2H-$\delta^{18}O$ slope than any bulk water resource.

Because our 2014 samples of stream water, groundwater, soil water, and stem water were distributed across the entire growing season and collected on a monthly basis, we were able to dissect the bulk data into snapshots in time and compare each date individually. In doing this, we found that during some sampling dates (July and August) the tree water does fall within the soil evaporation regression line, while in other months (May, June, September), they fall below (Figure 5).

The extreme enrichment of stem water in May 2014 can potentially be explained by tree phenology and post-winter xylem refilling. The trees were just beginning to bud and leaf out and were most likely just beginning to refill embolized vessels. Wintertime xylem embolism is often a result of freeze-thaw events during which insoluble gases as released from ice as bubbles, which may enlarge after thawing when tension is restored [Sperry and Sullivan 1992. It is probable that vapor water released in wintertime freeze-thaw cycles can diffuse across a vapor pressure gradient to the dry atmosphere. Additionally, the gradual evaporation of over-wintered stagnant water through the bark would cause isotopic enrichment of stem water [Phillips 1995]. Whether this enriched water persists in stem tissues is unknown, although dramatic isotopic shifts have been documented at the same time as leaf-out, suggesting that this artifact would be minimal after xylem refilling and not likely detected during continuous transpiration.

In early June 2014, the trees had fully expanded their leaves, but remained outside of the soil water domain (Figure 5). Upon comparison with soil moisture measurements (Figure 6, Table 3), June did not stand out as a particularly dry sampling date compared to subsequent months. During the July and August sampling dates where the trees align with the soil water regression line, there was not a clear pattern to implicate soil moisture availability. In July, soil moisture was still being continually depleted, while in August, the upper soil layers showed higher saturation due to a rain event (although below 5 cm, the soil remained dry).

However, in September when the trees were once again below the soil in dual-isotope space, the soils appeared equally as dry.

In addition, tree water potentials measured throughout the growing season did not indicate water stress. It is possible to experimentally generate “vulnerability curves” describing the potentials at which various degrees of cavitation occur for tree species. Vulnerability curves for boxelder [Hultine et al. 2008] and bigtooth maple [Taneda and Sperry 2008] suggest only minimal hydraulic conductivity loss due to cavitation for the levels measured in 2014.

Many have recently stressed the importance of using H and O isotopes together for the purposes of water resource identification [Meißner et al. 2014, McDonnell 2014], although most studies to date use only one or do not use them together. It is important to note that had this study been conducted using a single isotope rather than both δ^2H and δ^{18}O, the predicated water sources would be different depending on which isotope was measured (Figure 7). In fact, if using only δ^2H as in Dawson and Ehleringer [1991], the tree would appear to be using stream water, groundwater, and/or mid-depth soil water. However, if using only δ^{18}O, they appear to overlap with only shallow soil waters (Figure 4).

Several papers have recently reported similar results, citing the co-existence of multiple unmixed pools of water in the soil matrix, each held at different potentials and displaying distinct isotopic signatures. Chemical interactions within the soil matrix may further contribute fractionating effects that differ between the pools. In fact, over recent years, several studies have tried to extract water of known isotopic values that had previously been added to a variety of soil types. To date, none have been successful in the retrieval of similar isotope signatures in any soils of moderate or low moisture levels (Ingraham and Shadel 1992, Walker et al. 1994,

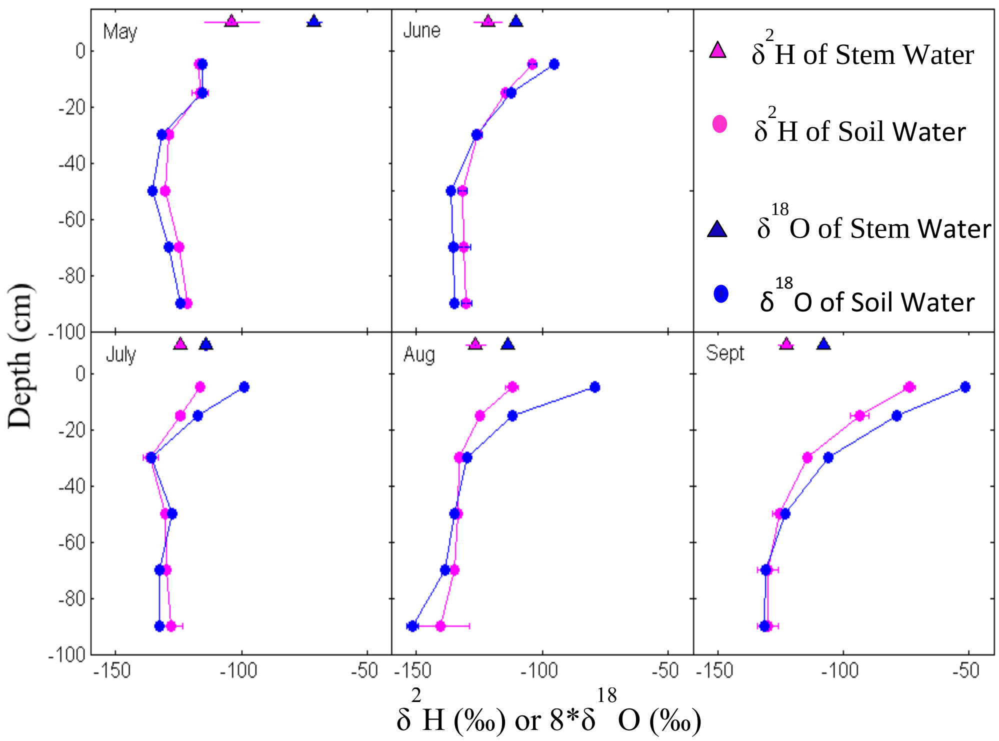

Figure 7: Soil depth profiles of δ^2H and $8*\delta^{18}O$, which would scale together if samples fell along the LMWL. Greater discrepancy between the profiles indicates greater *d-excess*. Average tree stem water isotopes (triangles) illustrate the inconsistency of rooting depth predictions when only using a single isotope for some months.

West et al. 2006, Orlowski et al. 2013, Meißner et al. 2014, Oerter et al. 2014, Stoll 2014). This is an indication that certain soil properties such as texture (clays and silts especially) as well as chemical constituents (e.g. cations and carbonate deposits) may preferentially exchange or interact with hydrogen and/or oxygen isotopes. This discrepancy highlights a gap in our knowledge of isotope interactions, illustrating either real soil/root water partitioning phenomena or single-isotope interactions that affect only hydrogen or oxygen.

It is also conceivable that these physical, mass dependent interactions occurring in small soil pores also occur within plant tissues. In this way, there may actually be multiple pools of water within the tree sapwood or heartwood that are isotopically distinct. The high degree of isotopic enrichment seen in the May 2014 tree stems may be evidence on wintertime water loss from the tree stem [Phillips and Ehleringer 1995]. This “overwintered” water may or may not be completely flushed out of the stems upon xylem refilling in the spring. If this water persists in either embolized vessels, axial parenchyma, nonconducting heartwood, or even in the most tissue-bound water, the overall isotopic signature of the tree xylem water will be a mixture of the current water resource and a past/evaporated one. Further, depending on the tree water status, transpiration rate, and conductivity, these smaller pools of water may constitute an exceedingly larger proportion of the extracted stem water, thus causing an apparent shift in isotope ratio.

The mechanism behind tree xylem evaporative enrichment at our site remains unclear; however, this is not a solitary outlier. In fact, studies from Oregon, Switzerland, China, and Mexico have also described this tree enrichment phenomenon, with no explanation [Brooks et al. 2010, Goldsmith et al. 2012, Singer et al. 2013, Zhao et al. 2013, Bertrand et al. 2014, Yang 2015]. Several recent studies may hint at important soil chemistry that may limit the value of isotopic analysis. At present, we do not have a method to extract water at only biologically significant water potentials. Cryogenic vacuum distillation extracts all water up to -15 MPa, far beyond a tree's tension capacity [McDonnell 2014]. Likewise, suction lysimeters can only access highly mobile pools of water, representing only a portion of tree accessible water [Landon et al. 1999, McDonnell 2014]. This study highlights a dilemma of stable isotope ecohydrology in which tree xylem water does not isotopically represent the trees' basic reservoir.

REFERENCES

Allison GB, Barnes CJ, Hughes MW, Leaney FWJ. 1984. Effect of climate and vegetation on oxygen-18 and deuterium profiles in soils. *Isotopes Hydrology, IAEA, Vienna* : 105-122.

Bertrand, G, Masini J, Goldscheider N, Meeks J, Lavastre V, Celle, Jeanton H, Hunkeler D. 2014. Determination of spatiotemporal variability of tree water uptake using stable isotopes ($\delta^{18}O$, $\delta^{2}H$) in an alluvial system supplied by a high altitude watershed, Pfyn forest, Switzerland. *Ecohydrology* **7**(2) : 319-333.

Biederman JA, Brooks PD, Harpold AA, Gochis DJ, Gutmann E, Reed DE, Pendall E, Ewers BE. 2014. Multiscale observations of snow accumulation and peak snowpack following widespread, insect-induced lodgepole pine mortality. *Ecohydrology* **7**: 150–162. doi: 10.1002/eco.1342.

Bond HW. 1977. Patterns of nutrient flux in a montane ecosystem. *Ph.D. University of Utah*.

Bowling DR, Massman WJ. 2011. Persistent wind-induced enhancement of diffusive CO_2 transport in a mountain forest snowpack. *Journal of Geophysical Research: Biogeosciences (2005–2012)* **116** (G4).

Brooks JR, Barnard HR, Coulombe R, McDonnell, JJ. 2010. Ecohydrologic separation of water between trees and streams in a Mediterranean climate. *Nature Geoscience* **3**(2) :100-104.

Brunel JP, Walker GR, Walker CD, Dighton JC, Kennett-Smith, A. 1991. Using stable isotopes of water to trace plant water uptake. *Stable isotopes in plant nutrition, soil fertility and environmental studies* : 543-51.

Brunel JP, Walker GR, Dighton JC, Monteny B. 1997. Use of stable isotopes of water to determine the origin of water used by the vegetation and to partition evapotranspiration. A case study from HAPEX-Sahel. *Journal of Hydrology* **188** : 466-481.

Clark ID, Fritz P. 1997. *Environmental Isotopes in Hydrogeology.* Lewis Publishers, New York..Colbeck SC. 1991. The layered character of snow covers. *Reviews of Geophysics* **29**(1) : 81-96.

Dawson TE, Ehleringer JR. 1991. Streamside trees that do not use stream water. *Nature* **350**(6316) : 335-337.

Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP. 2002. Stable isotopies in plant ecology. *Annual Review of Ecology and Systematics* **33** : 507-559.

Dawson TE, Pate JS. 1996. Seasonal water uptake and movement in root systems of Australian phraeatophytic plants of dimorphic root morphology: a stable isotope investigation. *Oecologia* **107**(1) : 13-20.

Dietermann N, Weiler M. 2013, Spatial distribution of stable water isotopes in alpine snow cover. *Hydrology and Earth System Sciences* **17** : 2657 – 2688.

Earman S, Campbell AR, Phillips FM, Newman BD. 2006. Isotopic exchange between snow and atmospheric water vapor: Estimation of the snowmelt component of groundwater recharge in the southwestern United States. *Journal of Geophysical Research: Atmospheres* (1984–2012) **111** : D9.

Ehleringer JR, Dawson TE. 1992. Water uptake by plants: perspectives from stable isotope composition. *Plant, Cell & Environment* **15**(9) : 1073-1082.

Ehleringer JR, Roden, J, Dawson T E. 2000. Assessing ecosystem-level water relations through stable isotope ratio analyses. In *Methods in ecosystem science.* Springer New York : 181-198.

Ellsworth PZ, Williams DG. 2007. Hydrogen isotope fractionation during water uptake by woody xerophytes. *Plant and Soil* **291**(1-2) : 93-107.

Feng X, Taylor S, Renshaw CE, Kirchner JW. 2002. Isotopic evolution of snowmelt 1. A physically based one-dimensional model. *Water Resources Research* **38**(10) : 35-1.

Friedman I, Benson C, Gleason J. 1991. Isotopic changes during snow metamorphism. *Stable Isotope Geochemistry: a Tribute to Samuel Epstein* **3** : 211-221.

Gat JR. 1996. Oxygen and hydrogen isotopes in the hydrologic cycle. *Annual Review of Earth and Planetary Sciences* **24**(1) : 225-262.

Gat JR, Confiantini R. 1981, Stable isotope hydrology, deuterium and Oxygen-18 in the water cycle, *International Atomic Energy Agency, Vienna*.

Goldsmith GR, Muñoz-Villers LE, Holwerda F, McDonnell JJ, Asbjornsen H, Dawson TE. 2012. Stable isotopes reveal linkages among ecohydrological processes in a seasonally dry tropical montane cloud forest. *Ecohydrology* **5**(6) : 773-790.

Gurney S, Lawrence D. 2004. Seasonal trends in the stable isotopic composition of snow and meltwater runoff in a subarctic catchment at Okstindan, Norway. *Nordic hydrology* **35**(2) : 119-137.

Gustafson JR, Brooks PD, Molotch NP, Veatch WC. (2010), Estimation of snow sublimation using natural chemical and isotopic tracers across a gradient of solar radiation, *Water Resources Research* **46** (12).

Hely AG, Mower RW, Harr CA. 1971. Water resources of Salt Lake County, Utah. *Utah Department of Natural Resources Technical Publication 31*.

Hultine KR, Bush SE, West AG, Burtch KG, Pataki DE, Ehleringer JR. 2008. Gender-specific patterns of aboveground allocation, canopy conductance and water use in a dominant riparian tree species: *Acer negundo*. *Tree Physiology* **28** : 1383-1394.

Ingraham NL, Shadel C. 1992. A comparison of the toluene distillation and vacuum/heat methods for extracting soil water for stable isotopic analysis. *Journal of Hydrology* **140**(1) : 371-387.

Jackson PC, Meinzer FC, Bustamante M, Goldstein G, Franco A, Rundel PW, Causin F. 1999. Partitioning of soil water among tree species in a Brazilian Cerrado ecosystem. *Tree physiology* **19**(11) : 717-724.

Kendall C, McDonnell JJ (Eds.). 1998. *Isotope tracers in catchment hydrology*. Elsevier.

Landon MK, Delin GN, Komor SC, Regan CP.1999. Comparison of the stable-isotopic composition of soil water collected from suction lysimeters, wick samplers, and cores in a sandy unsaturated zone. *Journal of Hydrology* **224**(1) : 45-54.

Lee J, Feng X, Faiia AM, Posmentier ES, Kirchner JW, Osterhuber R, Taylor S. 2010. Isotopic evolution of a seasonal snowcover and its melt by isotopic exchange between liquid water and ice. *Chemical Geology* **270**(1) : 126-134.

Lee J, Feng X, Posmentier ES, Faiia AM, Taylor S. 2009. Stable isotopic exchange rate constant between snow and liquid water. *Chemical Geology* **260**(1) : 57-62.

Li SG, Romero-Saltos H, Tsujimura M, Sugimoto A, Sasaki L, Davaa G, Oyunbaatar D. 2007. Plant water sources in the cold semiarid ecosystem of the upper Kherlen River catchment in Mongolia: A stable isotope approach. *Journal of Hydrology* **333**(1) : 109-117.

Maurer GE, Bowling DR. 2014. Seasonal snowpack characteristics influence soil temperature and water content at multiple scales in interior western US mountain ecosystems. *Water Resources Research* **50**(6) : 5216-5234.

McDonnell JJ. 2014. The two water worlds hypothesis: ecohydrological separation of water between streams and trees?. *Wiley Interdisciplinary Reviews: Water* **1**(4) : 323-329.

Meinzer FC, Andrade JL, Goldstein G, Holbrook NM, Cavelier J, Wright SJ. 1999. Partitioning of soil water among canopy trees in a seasonally dry tropical forest. *Oecologia* **121**(3) : 293-301.

Meißner M, Köhler M, Schwendenmann L, Hölscher D, Dyckmans J. 2014. Soil water uptake by trees using water stable isotopes (δ^2H and δ^{18}O)− a method test regarding soil moisture, texture and carbonate. *Plant and Soil* **376**(1-2) : 327-335.

Moser H, Stichler W. 1974. Deuterium and oxygen-18 contents as an index of the properties of snow covers. *International Association of Hydrological Sciences Publication* **114** : 122-135.

Oerter E, Finstad K, Schaefer J, Goldsmith GR, Dawson T, Amundson R. 2014. Oxygen isotope fractionation effects in soil water via interaction with cations (Mg, Ca, K, Na) adsorbed to phyllosilicate clay minerals. *Journal of Hydrology* **515** : 1-9.

Orlowski N, Frede HG, Bruggermann N, Breuer L. 2013. Validation and application of a cryogenic vacuum extraction system for soil and plant water extraction for isotope analysis. *Journal of Senors and Sensor Systems* **2** : 179.

Phillips SL, Ehleringer JR. 1995. Limited uptake of summer precipitation by bigtooth maple (*Acer grandidentatum* Nutt) and Gambel's oak (*Quercus gambelii* Nutt). *Trees* **9** : 214-219.

Scholander PF, Bradstreet ED, Hemmingsen EA, Hammel HT. 1965. Sap Pressure in Vascular Plants: Negative hydrostatic pressure can be measured in plants. *Science* **148** : 339-46.

Singer MB, Stella JC, Dufour S, Piegay H,Wilson RJS, Johnstone L. 2013. Contrasting water-uptake and growth responses to drought in co-occurring riparian tree species, *Ecohydrology* **6** (3) : 402-412.

Sokratov SA, Golubev VN. 2009. Snow isotopic content change by sublimation. *Journal of Glaciology* **55**(193) : 823-828.

Sommerfeld RA, Friedman I, Nilles M. 1987. The fractionation of natural isotopes during temperature gradient metamorphism of snow. In *Seasonal Snowcovers: Physics, Chemistry, Hydrology*: 95-105. Springer Netherlands.

Sperry JS, Sullivan JE. 1992. Xylem embolism in response to freeze-thaw cycles and water stress in ring-porous, diffuse-porous, and conifer species. *Plant Physiology* **100**(2) : 605-613.

Stichler W, Schotterer U, Fröhlich K, Ginot P, Kull C, Gäggeler H, Pouyaud B. 2001. Influence of sublimation on stable isotope records recovered from high-altitude glaciers in the tropical Andes. *Journal of Geophysical Research: Atmospheres (1984–2012)* **106**(D19) : 22613-22620.

Stoll M. 2014. H and O stable isotope compositions of different soil water types – effect of soil properties. (Masters Thesis) *Swedish University of Agricultural Sciences*.

Stratton LC, Goldstein G, Meinzer FC. 2000. Temporal and spatial partitioning of water resources among eight woody species in a Hawaiian dry forest. *Oecologia* **124**(3) : 309-317.

Taneda H, Sperry JS. 2008. A case-study of water transport in co-occurring ring- versus diffuse-porous trees: contrasts in water-status, conducting capacity, cavitation and vessel refilling. *Tree Physiology* **28** : 1641-1651.

Taylor S, Feng X, Kirchner JW, Osterhuber R, Klaue B, Renshaw CE. 2001. Isotopic evolution of a seasonal snowpack and its melt. *Water Resources Research* **37**(3) : 759-769.

Walker GR, Woods PH, Allison GB. 1994. Interlaboratory comparison of methods to determine the stable isotope composition of soil water. *Chemical Geology* **111**(1) : 297-306.

Weltzin JF, McPherson GR. 1997. Spatial and temporal soil moisture resource partitioning by trees and grasses in a temperate savanna, Arizona, USA. *Oecologia* **112**(2) : 156-164.

West AG, Patrickson SJ, Ehleringer JR. 2006. Water extraction times for plant and soil materials used in stable isotope analysis. *Rapid Communication in Mass Spectrometry* **20** : 1317-1321.

White JW, Cook ER, Lawrence JR. 1985. The DH ratios of sap in trees: Implications for water sources and tree ring DH ratios. *Geochimica et Cosmochimica Acta* **49**(1) : 237-246.

Yang B, Wen X, Sun X. 2015. Seasonal variations in depth of water uptake for a subtropical coniferous plantation subjected to drought in an East Asian monsoon region. *Agricultural and Forest Meteorology* **201** : 218-228.

Zencich SJ, Froend RH, Turner JV, Gailitis V. 2002. Influence of groundwater depth on the seasonal sources of water accessed by Banksia tree species on a shallow, sandy coastal aquifer. *Oecologia* **131**(1) : 8-19.

Zhou S, Nakawo M, Hashimoto S, Sakai A. 2008. Preferential exchange rate effect of isotopic fractionation in a melting snowpack. *Hydrological Processes* **22**(18) : 3734-3740.

Zhao P, Tang X, Zhao P, Wang C, Tang J. 2013. Identifying the water source for subsurface flow with deuterium and oxygen-18 isotopes of soil water collected from tension lysimeters and cores. *Journal of Hydrology* **503** : 1-10.